1-1-1991

Fertility and chromosome number in interspecific Impatiens hybrids

Robin Lynn Fruth
Iowa State University

Fertility and chromosome number in interspecific *Impatiens* hybrids

by

Robin Lynn Fruth

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE
Major: Horticulture

Iowa State University
Ames, Iowa

1991

TABLE OF CONTENTS

ABSTRACT

The effects of chromosome number on female fertility of interspecific hybrids among Impatiens from Java (I. platypetala Lindl.), New Guinea (I. hawkeri Bull.), and Celebes (I. aurantiaca Teysm.) and the role of unreduced gametes in determining chromosome number and fertility were investigated. Two 24-chromosome interspecific hybrids were used as parents, 'Tangeglow' and '7851-1'. The F_1 offspring from this cross had chromosome numbers ranging from 28 to 36. Because 'Tangeglow' is seed-propagated and has normal meiosis, '7851-1' must have produced some male gametes that were incompletely unreduced and other gametes that were completely unreduced. The female fertility of interspecific hybrids with 36 chromosomes and 29 chromosomes was compared. The hybrids with 36 chromosomes exhibited greater fertility than the hybrids with 29 chromosomes. These results indicate that completely unreduced gametes provide for greater female fertility in the hybrid progeny because of the presence of complete genomes of Java and New Guinea Impatiens.

I. INTRODUCTION

New Guinea Impatiens cultivars, I. hawkeri Bull., have become a big part of the bedding plant industry since their introduction in the 1970's (USDA, 1972; Winters, 1973). Their foliage variegation and wide flower color range is of great ornamental value, but they are susceptible to hot, windy conditions found in the Midwestern United States (Pasutti and Weigle, 1980). The Impatiens from Java, I. platypetala Lindl., and Celebes, I. aurantiaca Teysm., have exhibited increased heat-tolerance compared with New Guinea cultivars. Various interspecific hybrids have been produced, but sterility has been a continuous problem (Arisumi, 1973a,b, 1974, 1975, 1978, 1980; Armstrong, 1976; Beck et al, 1974; Pasutti and Weigle, 1980; Pasutti et al., 1977; Weigle et al., 1979; Weigle and Pasutti, 1979; Weigle, 1979). Production of amphidiploids overcomes the problem of sterility, but makes selection for heat tolerance more tedious. Some naturally occurring fertility has been found by crossing Impatiens 'Tangeglow' with a New Guinea x Java hybrid, both parents being 24-chromosome interspecific hybrids (Stephens et al., 1988). The nature of this fertility was investigated in this study.

Past researchers have suggested that interspecific hybrids have the ability to produce unreduced gametes, which seem to be more functional (Hermsen, 1984a,b). The formation

of unreduced gametes has been observed in interspecific hybrids of blueberries (Dweikat and Lyrene, 1988), morning glories (Eckenwalder and Brown, 1986), cassava (Hahn et al., 1990), brassicas (Heyn, 1977; Vesperinas, 1988), and potato (Lam, 1974). Mechanisms for the formation of the unreduced gametes also have been suggested for the preceding crops. These mechanisms vary with different species.

Pasutti (1977) and Pasutti et al. (1977) also studied the 'Tangeglow' x (I. hawkeri x platypetala) cross, but with a New Guinea x Java hybrid that was different from the one used in this study. Because of very poor pollen fertility in this hybrid, Pasutti (1977) believed that those few functional male gametes that were formed occurred because of the random-assortment principle , which states that an entire genome of a hybrid or the parental genomes could migrate to a pole creating stable gametes. The frequency of stable gametes would be $(\frac{1}{2})^n \times 3$ where n equals the number of chromosomes present in the hybrid (Srb et al., 1965).

The objectives of this study were to investigate the nature of pollen fertility in a New Guinea x Java hybrid and to determine the effect of chromosome number on female fertility in interspecific hybrids composed of genomes from New Guinea, Java, and Celebes Impatiens.

II. MATERIALS AND METHODS

A. Description of Plant Material

Plants used in the intial cross were 'Tangeglow' and '7851-1'. There have been differing opinions as to the parentage of 'Tangeglow', which has been shown to have 24 chromosomes (Pasutti and Weigle, 1980). One hypothesis is that 'Tangeglow' is a New Guinea x Celebes ('Tangerine') hybrid (Armstrong, 1976). It was found as a fertile sport on a 2n=20 plant, which means that in order for 'Tangeglow' to have a chromosome number of 2n=24 the Celebes genome must have doubled. The gametes would have 4 Celebes chromosomes and 8 New Guinea chromosomes. The other hypothesis is that 'Tangeglow' is a Java x Celebes hybrid with the entire genome doubled (Pasutti, 1977). A doubling of the entire genome seems more likely to produce fertile gametes. The gametes would have 4 Celebes chromosomes and 8 Java chromosomes. Celebes chromosomes are twice as large as New Guinea and Java chromosomes. Both parents contributed 4 Celebes chromosomes and 8 Java or New Guinea chromosomes. However, the size difference between New Guinea and Java chromosomes is not big enough to determine which species contributed the chromosomes present in 'Tangeglow' gametes. No matter which is the true parent, normal meiosis has been assumed in 'Tangeglow' because several selfed generations show only minimal phenotypic variation in the progeny and no variation in the 2n=24

chromosome number (Pasutti, 1977). The hybrid '7851-1' is a 24-chromosome male-fertile, female-sterile plant derived from a New Guinea x Java cross (Hicks et al., 1987).

Six of the 15 F_1 progeny from the 'Tangeglow' x '7851-1' cross were selected for the female fertility study: 3 genotypes with 36 chromosomes ('88-1-5', '88-1-6', and '88-1-13') and 3 genotypes with 29 chromosomes ('88-1-3', '88-1-10', and '88-1-11'). A pedigree for the 6 selected F_1 plants and the backcross offspring from these 6 plants is presented in Figure 1. Only 4 BC_1F_1 offspring were obtained from the backcrosses of the F_1 genotypes with 29 chromosomes. These crosses typically had low seed set and the hybrid seed had poor germination. Three seedlings were obtained from '88-1-11' x '7851-1' backcross and 1 seedling from '88-1-3' x '7851-1' backcross. Forty-two BC_1F_1 offspring were obtained from the backcrosses of the F_1 genotypes with 36 chromosomes: 10 from '88-1-5' x '7851-1' backcross, 17 from '88-1-6' x '7851-1' backcross, and 15 from '88-1-13' x '7851-1' backcross.

B. Numbering System

Plants were numbered to facilitate record keeping using a system developed at the University of Minnesota. The first number specifies the year, the second number the cross number, and the third number the offspring number.

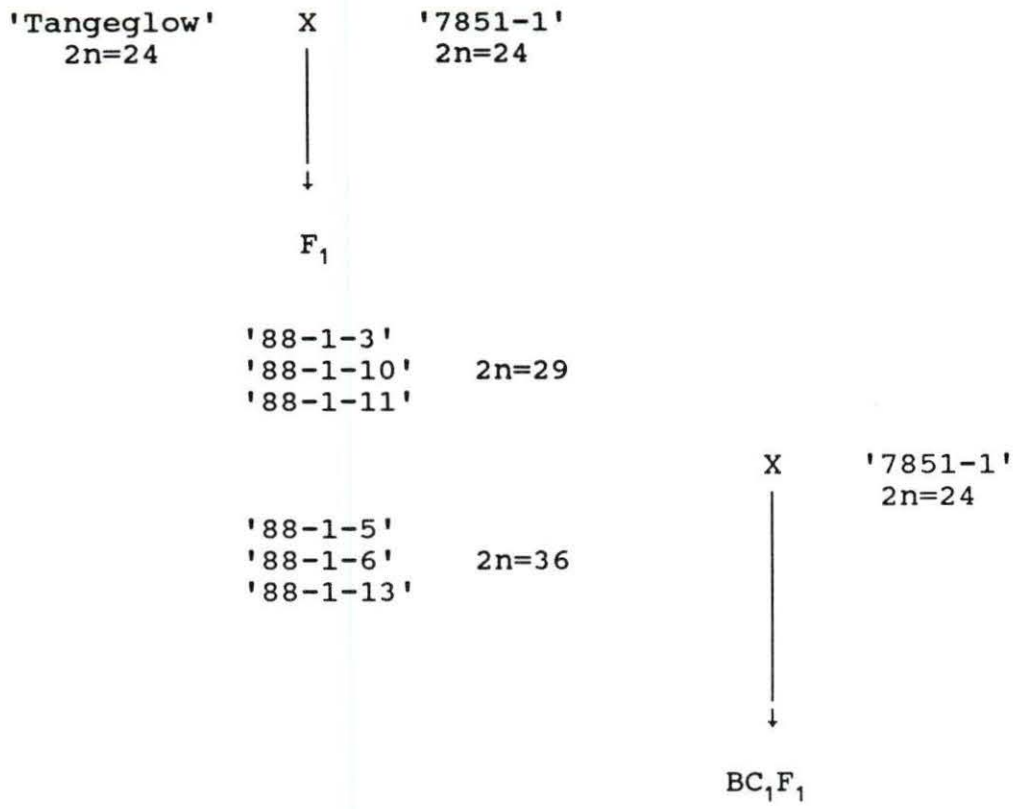


Figure 1. Pedigree for the interspecific Impatiens hybrids (88-1's) and the backcross progenies that were used for this study

C. Pollination and Seed Collection

Pollen was transferred from the anther hood to the receptive stigma. No emasculation was required because pollen was shed before the stigma became receptive. The developing flower pod was bagged during the fourth week after pollination to collect the seed when the pod burst. The seed was allowed to air dry for 2-3 days before being stored in an envelope until being planted.

D. Chromosome Counts

Root tip preparation

Root tips were prepared using the same procedure as Pasutti (1977), which was originally developed by Palmer and Heer (1973) for soybeans. Pasutti's procedure is as follows with a few modifications. Cuttings were taken from plants and rooted in perlite under mist for about 2 weeks. Healthy root tips, which were white with a brown root cap, were taken and placed in saturated paradichlorobenzene for 3 hours at room temperature, which was later modified to 6 hours for the genotypes with 36 chromosomes to obtain maximum chromosome contraction. After the 3 or 6 hour treatment, root tips were fixed in a 3:1 fixative (95% ethanol:glacial acetic acid) for a minimum of 20 minutes at room temperature. Fixed root tips were hydrolyzed in 1N HCL at 60°C for 10-15 minutes. The hydrolyzed root tips were rinsed with distilled water and

placed in Leuco-basic fuchsin stain for 90 minutes. The root tips then were placed in cold distilled water (4°C) for 5 minutes before transferring to a spot plate. The excess water in the spot plate was soaked up before covering the root tips with pectinase solution. The spot plate was covered with foil and incubated at 30°C for 60 minutes, which was later modified to 50 minutes because the root tips were too soft at the longer time. The root tips were removed from the spot plate, rinsed with cold distilled water (4°C), and stored in 70% ethanol at 4°C. The tip of the root (1mm) was placed on a slide with a drop of aceto-carmine stain, covered with a cover slip, heated slowly over a flame to set the stain, and then tapped lightly to spread out the cells for observation. Saturated paradichlorobenzene, pectinase, leuco-basic fuchsin stain, and aceto-carmine were prepared according to Pasutti (1977).

Photographs and Interpretive Drawings

Photos for documenting chromosome counts were taken using an Olympus light microscope with a 35mm Nikon camera. All photos were taken using 1000x magnification under oil. Additional enlargement during printing increased the magnification. Kodak technical pan film 2415 was used with a green filter using the automatic exposure meter. Cells with the highest number of chromosomes in the same focal plane were photographed. When a series of photos was taken, tracings of

the photos were made to bring the focal planes together.

Confirmation of a Count

Confirmation of the chromosome number of a genotype was done by focussing on different planes while viewing the cell through the microscope. A chromosome count for a given genotype was considered to be confirmed when 3 or more of the cells displayed a common chromosome number.

E. Experimental Design and Data Collection

Female Fertility Experiment

This experiment was designed to determine the effects of chromosome number on the percent fertilized ovules, which was measured by the number of fertilized ovules per ovary expressed as a percent of total ovules per ovary. The experiment consisted of 6 treatments (the 6 F_1 genotypes from the 'Tangeglow' x '7851-1' cross), 3 seasons (summer, fall, and winter 1990), and 5 blocks per season, each block being a particular day. Each genotype was pollinated 2 or 3 times on one day. Two weeks later the ovaries were viewed under the dissecting microscope. The total number of ovules was counted along with the number of fertilized ovules and these data were converted into percentage values to be analysed upon completion of the experiment.

Seed Set Experiment

This experiment was designed to determine the effect of chromosome number on the mean number of mature seeds per ovary. Two to 3 pollinations were made to each of the 6 F_1 genotypes at one time and allowed to mature (about 4 weeks). The seeds were collected and counted, and the total number of ovules was determined for each ovary. Both of these data sets were analysed. The successful pollination percentage for each genotype was calculated from the number of ovaries containing mature seed, based on the total number of pollinations attempted. The collected seeds were planted to determine the percent germination for each genotype.

F. Data Analysis

The data for percent fertilized ovules were analysed as an incomplete block design because of the abortion of some of the ovaries. The data for seed set and total number of ovules per ovary were analysed as a completely randomized design. A single degree-of-freedom F-test contrasting the 36-chromosome genotypes and the 29-chromosome genotypes was performed in each analysis. All statistical analyses used the Statistical Analysis System's General Linear Model (Barr et al., 1976).

III. RESULTS

The 15 F_1 progeny from the 'Tangeglow' x '7851-1' cross had chromosome numbers that ranged from 28 to 36: 5 with 36 chromosomes, 1 with 34, 1 with 32, 2 with 30, 3 with 29, and 2 with 28 chromosomes (Table 1). No confirmed counts were obtained for plant '88-1-12' because of its lack of vigor and poor quality root tips.

Root tip squashes of '7851-1' and 'Tangeglow' showed that both '7851-1' and 'Tangeglow' have 24 chromosomes (Figure 2). For 'Tangeglow', 22 chromosomes are in focus and 2 out of focus, in a different plane. These 2 chromosomes are indicated on the interpretive drawing by open circles (Figure 2). Genotypes '88-1-5', '88-1-6', and '88-1-13' have been confirmed as having 36 chromosomes, whereas genotypes '88-1-3', '88-1-10', and '88-1-11' have been confirmed as having 29 chromosomes (Figure 3).

Chromosome number had significant effects on the mean percent fertilized ovules. The genotypes with 36 chromosomes exhibited greater female fertility than the genotypes with 29 chromosomes (Figure 4). A single degree-of-freedom F-test contrasting these 2 groups was significant at the P=0.01 level.

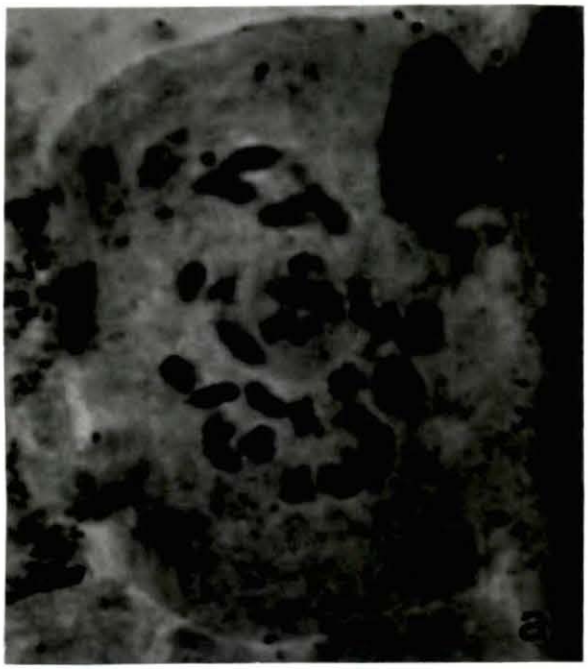
Chromosome number also was shown to have effects on the mean number of ovules per ovary, the mean number of mature seeds per ovary, the successful pollination percentage, and

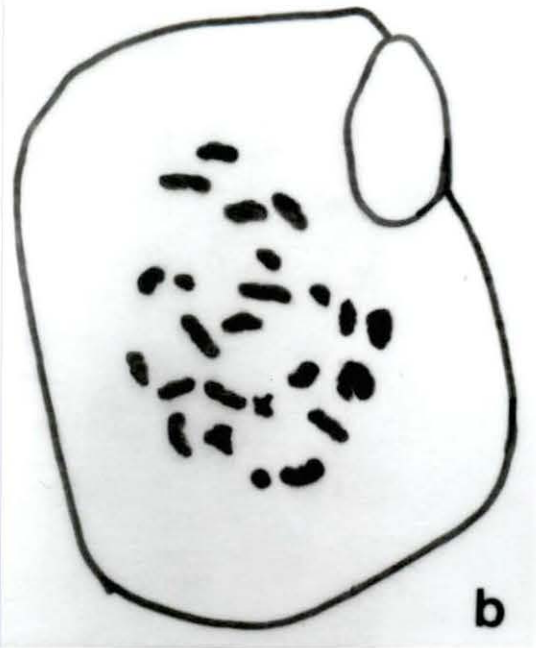
the germination percentage. The genotypes with 36 chromosomes produced more ovules per ovary and seeds per ovary than the genotypes with 29 chromosomes (Table 2). Single degree-of-freedom F-tests contrasting these 2 groups for mean number of ovules per ovary and mean number of seeds per ovary were significant at the P=0.01 level. The successful pollination percentage was higher for genotypes with 36 chromosomes than for genotypes with 29 chromosomes (Table 2). The successful pollination percentage essentially measures the abortion rate because 1 fertilized ovule per ovary must be present to prevent the ovary from abscising. The abortion rate of '88-1-11' was more like that of genotypes with 36 chromosomes than genotypes with 29 chromosomes, but the mean number of mature seeds collected per successful pollination from '88-1-11' was significantly fewer than genotypes with 36 chromosomes (Table 2). The percent seed germination also differed between each group of genotypes, with the 29-chromosome genotypes exhibiting lower germination than the 36-chromosome genotypes (Table 2).

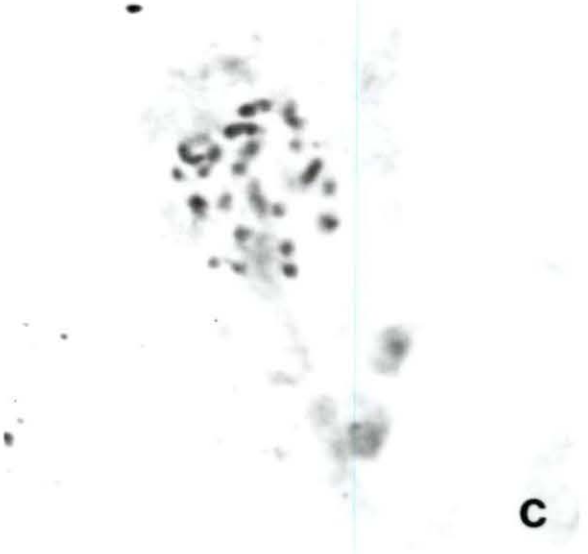
Table 1. Chromosome numbers for 14 of 15 F_1 progeny of a 'Tangeglow' x '7851-1' cross

Genotype	Chromosome number
88-1-1	36
88-1-2	34
88-1-3	29
88-1-4	30
88-1-5	36
88-1-6	36
88-1-7	28
88-1-8	30
88-1-9	28
88-1-10	29
88-1-11	29
88-1-12	unknown
88-1-13	36
88-1-14	32
88-1-15	36

Figure 3. a) Root tip squash of '88-1-6' b) Tracing of the '88-1-6' cell showing 36 chromosomes c, d, e) Root tip squash of '88-1-10' focussing on the upper, middle, and lower chromosomes, respectively f) Tracing of the '88-1-10' cell showing 29 chromosomes (x 1000)


a


b


c

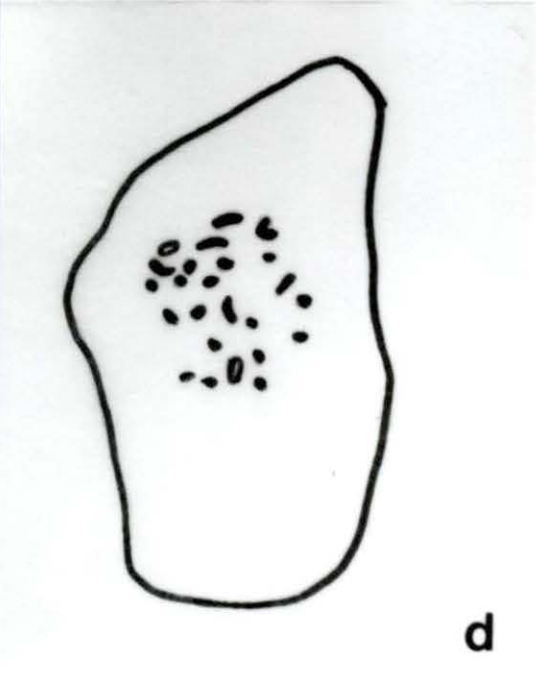
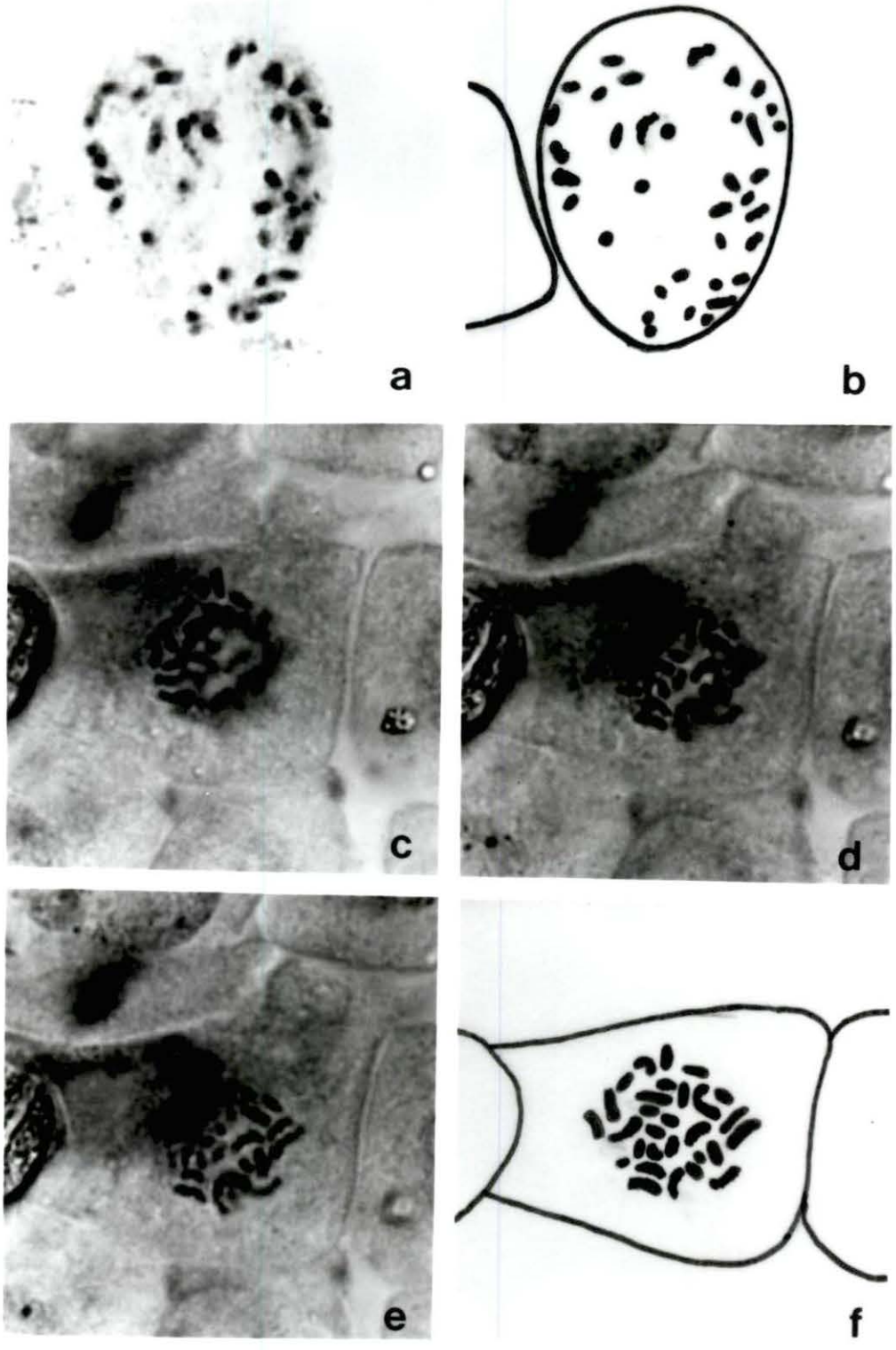

d

Figure 2. a) Root tip squash of '7851-1' b) Tracing of the '7851-1' cell showing 24 chromosomes c) Root tip squash of 'Tangeglow' d) Tracing of the 'Tangeglow' cell showing 24 chromosomes. (x 1000)


a
b
c
d
e
f

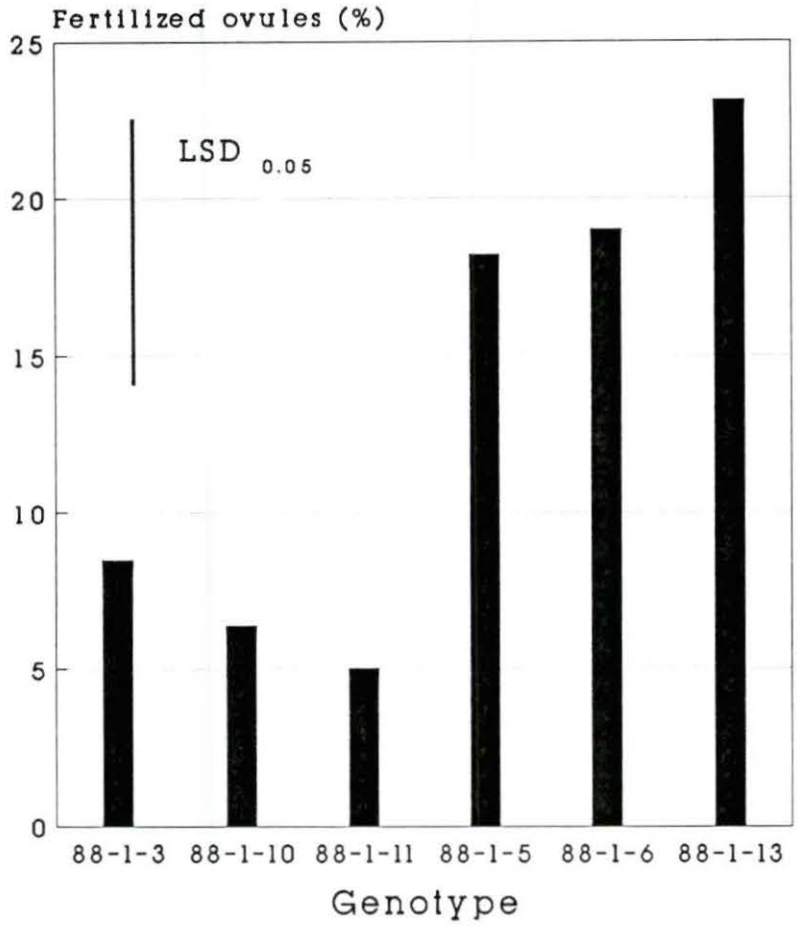


Figure 4. Effect of chromosome number on the mean % fertilized ovules for selected interspecific Impatiens hybrids (88-1-3, -10, -11, -5, -6, and -13). The data were collected two weeks after pollination. The number of fertilized ovules per ovary was expressed as a percent based on the number of total ovules per ovary. A single degree-of-freedom F-test contrasting plants with 36 chromosomes (88-1-5, -6, and -13) and plants with 29 chromosomes (88-1-3, -10, and -11) was significant at the P=0.01 level

Table 2. Effect of chromosome number on the successful pollination percentage, the mean number of ovules per ovary, the mean number of mature seeds per ovary, and the germination percentage

Genotype	Successful pollination (%)	Mean No. ovules/ ovary[a]	Mean No. seeds/ ovary[a]	Germination of seed (%)
88-1-3	10	34	2	16
88-1-10	10	35	1	0
88-1-11	52	41	2	33
88-1-5	86	45	7	67
88-1-6	82	42	8	73
88-1-13	73	40	8	47
$LSD_{0.05}$		9	3	

[a]A single degree-of-freedom F-test contrasting 36-chromosome genotypes and 29-chromosome genotypes was significant at the P=0.01 level.

IV. DISCUSSION

Thirty-six percent of the F_1 progeny were observed as having 36 chromosomes which suggests that gametes from '7851-1' must have been unreduced gametes. Under normal circumstances we would expect to get plants with 24 chromosomes but 28 was the lowest number observed. Mok and Peloquin (1975a,b,c) and Mok et al. (1975) have reported three mechanisms for unreduced pollen formation in diploid potato: parallel spindle formation, premature cytokinesis I, and premature cytokinesis II. Pasutti (1977) and Pasutti et al. (1977) believed that random assortment was occurring during the formation of gametes and in order for a gamete to be stable the entire genome or the parental genomes of the hybrid must migrate to one pole. In the case of '7851-1' the frequency of this occurring would be 3 gametes out of 16,777,186 $[(\frac{1}{2})^{24} \times 3]$. According to this principle the progeny from the 'Tangeglow' x '7851-1' cross would then have chromosome numbers of 20, 28, and 36. Each progeny would receive 12 chromosomes from 'Tangeglow' and the 8 Java chromosomes from '7851-1' to total 20, the 16 New Guinea chromosomes from '7851-1' to total 28, or all 24 chromosomes from '7851-1' to total 36. In this study, no progeny with less than 28 chromosomes were obtained and 7 of the 15 F_1 progeny had chromosome numbers other than 28 or 36. Therefore, the available evidence indicates that '7851-1' is

producing unreduced gametes and partially unreduced gametes. Tetrads, triads, and dyads have all been observed in pollen-mother-cell squashes of '7851-1' (unpublished data) which would indicate different mechanisms for the formation of unreduced gametes and partially unreduced gametes. One possibility is the reduction or absence of homologous pairing due to the lack of homology between chromosomes from different species. The lack of pairing may stimulate the formation of restitution nuclei and 2n-gametes (Hermsen, 1984a). Unreduced gametes could be obtained from such a mechanism, but in order to get partially unreduced gametes from this mechanism, there would have to be lagging chromosomes during the second division.

Chromosome number affected female fertility of interspecific Impatiens hybrids in this study. One important consideration is the composition of each of the 29-chromosome hybrids. In order to get 29 chromosomes there must have been 7 lagging chromosomes during metaphase II in '7851-1' gamete formation. If the 7 lagging chromosomes were at random, than presumably different genotypes would have had different lagging chromosomes. This would suggest that the 29-chromosome plants do not have the same genomic composition, whereas the 36-chromosome plants would have the same genomic composition: 16 New Guinea chromosomes, 16 Java chromosomes, and 4 Celebes chromosomes. The 29-chromosome genotypes would

presumably be lacking chromosomes of New Guinea or Java or both totaling 7 chromosomes, which could reduce pairing among chromosomes of a genome in meiosis of these hybrids, leading to a reduction in female fertility.

Genotype '88-1-11', a 29-chromosome plant, was somewhat of an exception in that this genotype had a lower abortion rate and a higher germination percentage than the other two 29-chromosome plants, genotypes '88-1-3 and '88-1- 10'. Phenotypically '88-1-11' resembled the 36-chromosome plants, having dark orange flower color and large flower size, whereas '88-1-3' and '88-1-10' had a faded orange flower color and smaller flower size. One possibility is that different chromosomes were lagging behind during metaphase II and the absence of certain chromosomes could have caused the increased abortion in '88-1-3'and '88-1-10'.

LITERATURE CITED

Arisumi, T. 1973a. Chromosome numbers and interspecific hybrids among New Guinea Impatiens species. J. Hered. 64:77-79.

Arisumi, T. 1973b. Morphology and breeding behavior of colchicine-induced polyploid Impatiens spp. L. J. Amer. Soc. Hort. Sci. 98:599-601.

Arisumi, T. 1974. Chromosome numbers and breeding behavior of hybrids among Celebes, Java, and New Guinea species of Impatiens L. Hortscience 9:478-479.

Arisumi, T. 1975. Phenotypic analysis of artificial and natural amphiploid cultivars of New Guinea and Indonesian species of Impatiens L. J. Amer. Soc. Hort. Sci. 100:381-383.

Arisumi, T. 1978. Hybridization among diploid and tetraploid forms of New Guinea, Java, and Celebes Impatiens spp. J. Amer. Soc. Hort. Sci. 103:355-361.

Arisumi, T. 1980. Chromosome numbers and comparative breeding behavior of certain Impatiens from Africa, India, and New Guinea. J. Amer. Soc. Hort. Sci. 105:99-102.

Armstrong, R. J. 1976. Introduction and breeding of the New Guinea Impatiens. AABGA Bull. 1974:36-38.

Barr, A. J., J. H. Goodnight, J. P. Sall, and J. T. Helwig. 1976. A users guide to SAS. Sparks Press, Raleigh, N.C.

Beck, A. R., J. L. Weigle, and E. W. Kruger. 1974. Breeding behavior and chromosome numbers among New Guinea and Java Impatiens species, cultivated varieties, and their interspecific hybrids. Can. J. Bot. 52:923-925.

Dweikat, I. M. and P. M. Lyrene. 1988. Production and viability of unreduced gametes in triploid interspecific blueberry hybrids. Theor. Appl. Genet. 76:555-559.

Eckenwalder, J. E. and B. P. Brown. 1986. Polyploid speciation in hybrid morning glories of Ipomoea L. sect. Quamoclit Griseb. Can. J. Genet. Cytol. 28:17-20.

Hahn, S. K., K. V. Bai, and R. Asiedu. 1990. Tetraploids, triploids, and 2n pollen from diploid interspecific crosses

with cassava. Theor. Appl. Genet. 79:433-439.

Hermsen, J. G. Th. 1984a. Mechanisms and genetic implications of 2n-gamete formation. Iowa State J. Research 58:421-434.

Hermsen, J. G. Th. 1984b. The potential of meiotic polyploidization in breeding allogamous crops. Iowa State J. Research 58:435-448.

Heyn, F. W. 1977. Analysis of unreduced gametes in the Brassiceae by crosses between species and ploidy levels. Z. Pflanzenzuchtg. 78:13-30.

Hicks, C. B., L. C. Stephens, and J. L. Weigle. 1987. In vitro pollen germination and viability of a Java x New Guinea Impatiens interspecific hybrid. Can. J. Bot. 65:1967-1968.

Lam, S. 1974. Origin and formation of unreduced gametes in the potato. J. Hered. 65:175-178.

Mok, D. W. S., and S. J. Peloquin. 1975a. Three mechanisms of 2n pollen formation in diploid potatoes. Can. J. Genet. Cytol. 17:217-225.

Mok, D. W. S., and S. J. Peloquin. 1975b. The inheritance of three mechanisms of diplandroid (2n pollen) formation in diploid potatoes. Heredity 35:295-302.

Mok, D. W. S., and S. J. Peloquin. 1975c. Breeding value of 2n pollen (diplandroids) in tetraploid X diploid crosses in potatoes. Theor. Appl. Genet. 46:307-314.

Mok, D. W. S., S. J. Peloquin, and T. R. Tarn. 1975. Cytology of potato triploids producing 2n pollen. Amer. Potato J. 52:171-174.

Palmer, R. G. and H. Heer. 1973. A root tip squash technique for soybean chromosomes. Crop Sci. 13:389-391.

Pasutti, D. W. 1977. M.S. Thesis. Iowa State University. Ames, Iowa.

Pasutti, D. W. and J. L. Weigle. 1980. Pollen fertility in Java X New Guinea Impatiens interspecific hybrids. Can. J. Bot. 58:384-387.

Pasutti, D. W., J. L. Weigle, and A. R. Beck. 1977. Cytology and breeding behavior of some Impatiens hybrids and their

backcross progeny. Can. J. Bot. 55:296-300.

Srb, A. M., R. D. Owen, and R. S. Edgar. 1965. General Genetics. The W. H. Freeman and Company, San Francisco. pp.217-218.

Stephens, L. C., J. L. Weigle, and S. L. Krell. 1988. Flower color inheritance in inbred progenies of Impatiens interspecific hybrids. J. Hered. 79:136-137.

United States Department of Agriculture. 1972. Notice to plant breeders and nurserymen relative to the release of 23 Impatiens introductions. U.S.D.A. Agricultural Research Service,Plant Science Research Division, Beltsville, Maryland.

Vesperinas, E. S. 1988. Obtainment of some new intergeneric and interspecific hybrids between wild Brassiceae. Candollea 43:499-504.

Weigle, J. L. 1979. 'Tropical Sunset' Impatiens. Hortscience 14:767.

Weigle, J. L. and D. W. Pasutti. 1979. 'Blue Moon' Impatiens. Hortscience 14:766.

Weigle, J. L., D. W. Pasutti, and A. R. Beck. 1979. 'Burgundy' Impatiens. Hortscience 14:768.

Winters, H. F. 1973. New Impatiens from New Guinea. Amer. Hort. 52:16-22

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my advisor, Dr. Loren Stephens, for his guidance, encouragement, and patience. I also would like to express my appreciation to my other committee members, Dr. Reid Palmer and Dr. Rick Hall, for their valuable time and suggestions. I thank fellow graduate students; Jeff Ryan, Dianna Liu, and Kim Klock; for their friendship. I also thank family and friends in Minnesota and Wisconsin for their love, encouragement, and assistance with Briant. And finally, I dedicate this manuscript to my two-year-old son, Briant.